(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

PCT

(43) International Publication Date
15 June 2006 (15.06.2006)

(10) International Publication Number
WO 2006/061643 A1

(51) International Patent Classification:
C12N 15/869 (2006.01) *A61K 39/245* (2006.01)

(21) International Application Number:
PCT/GB2005/004754

(22) International Filing Date:
9 December 2005 (09.12.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0427001.3 9 December 2004 (09.12.2004) GB

(71) Applicant *(for all designated States except US)*: **OXXON THERAPEUTICS LIMITED** [GB/GB]; 2nd Floor Park Gate, 25 Milton Road, Oxford, Oxfordshire OX14 4SH (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ANDERSON, Richard** [GB/GB]; 2nd Floor Florey House, 3 Robert Robinson Park, Oxford Science Park, Oxford OX4 4GP (GB). **HOWARD, Michael, Keith** [GB/AT]; Baxter AG, Biomedical Research Center, A-2304 Orth/Donau, Uferstr. 15 (AT). **SCHNEIDER, Joerg** [DE/GB]; 2nd Floor Florey House, 3 Robert Robinson Park, Oxford Science Park, Oxford OX4 4GP (GB). **VAN MAURIK, Andre** [NL/GB]; 2nd Floor Florey House, 3 Robert Robinson Park, Oxford Science Park, Oxford OX4 4GP (GB).

(74) Agents: RICHARDS, William et al.; D Young & Co, 120 Holborn, London EC1N 2DY (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR VACCINATING USIGN A PRIME-BOOST REGIME AND HSV AS A VECTOR

(57) Abstract: The invention provides a method for vaccinating a subject against a target pathogen or disease, comprising administering at least one dose of: (a) a first, priming, composition comprising at least one immunogenic epitope associated with the target pathogen or disease, and (b) a second, boosting, composition comprising at least one immunogenic epitope associated with the target pathogen or disease, including at least one immunogenic epitope which is the same immunogenic epitope as the first composition, wherein at least one of the first and second composition comprises a nucleic acid encoding said immunogenic epitope in a replication impaired HSV vector, provided that the vector used in the first composition is different to the vector used in the second composition.

WO 2006/061643 A1

B16

METHOD FOR VACCINATING USING A PRIME-BOOST REGIME AND HSV AS A VECTOR

The present invention relates to herpesvirus vectors. In particular, the present invention relates to attenuated herpesvirus vectors such as DISC herpesvirus vectors for use in prime-boost immunisation.

The present invention also relates to prime-boost vaccination methods using herpesviral vectors, in particular heterologous prime-boost vaccination regimes employing two different non-replicating viral vector compositions, one of which is a herpesvirus vector.

BACKGROUND

Prime-boost vaccination strategies have been proposed in the art. In general, prime-boost vaccination has been attempted using naked DNA and poxvirus-based vaccines (see EP 0979284).

The most used poxvirus for vaccination is vaccinia virus, which was used for vaccination against smallpox. In common with the other poxviruses, vaccinia virus resides within the cell cytoplasm where it expresses the proteins needed for viral replication. Recombinant vaccinia can, therefore, deliver foreign antigens to the cytoplasm of mammalian cells, thereby allowing them direct access to antigen processing pathways which leads to presentation of antigen derived peptides in association with MHC Class I and Class II molecules on the cell surface (Moss, 1991, *Proc Natl Acad Sci USA 93*, 11341-8). This property makes vaccinia useful as recombinant vaccines, particularly for stimulating CD8+ and CD4+ T cell immune responses.

Concern about the capacity of vaccinia virus to replicate in mammalian cells has limited its clinical use and led to the search for safer alternatives. These include attenuated vaccinia viruses, such as modified vaccinia virus Ankara (MVA) (Meyer *et al*, 1991, *J Gen Virol 72*, 1031-8; Sutter and Moss, 1992, *Proc Natl Acad Sci USA 89*, 10847-51; Sutter *et al*, 1994, *Vaccine 12*, 1032-40), which undergoes limited replication in human

cells (Blanchard *et al*, 1998, *J Gen Virol 79*, 1159-67), and the avipoxviruses, such as fowlpox, which do not proliferate in mammalian cells (Somogyi *et al*, 1993, *Virology 197*, 439-44).

Herpesviruses have been proposed for use in prime-boost applications as priming agents (see WO0224224) and more generally (see WO0044410). However, herpesviruses have never been tested in prime-boost vaccination and their effectiveness has never been demonstrated. Herpes Simplex virus (HSV) is an enveloped, icosahedral and double stranded DNA virus large enough to encode roughly 70 transcripts of which only half are required for viral replication. This suggests ample space for recombinant gene insertions and the delivery of multiple genes in one vector. Like other large DNA viruses, HSV has also evolved a variety of mechanisms to evade the host immune responses to infection. The Fc and complement receptors on the surface of HSV weaken humoral responses while its cell to cell spread and latency in neurons facilitate the evasion of antibody neutralisation and clearance. HSV is also capable of inhibiting antigen presentation to CD8 cells by blocking the viral peptide presentation by MHC class I molecules on the surface of infected cells. HSV possesses a number of valuable characteristics such as a high transduction efficiency, the ability to infect post-mitotic cells and has a large transgenic capacity.

Attenuated HSV vectors are known, which do not replicate in host cells. However, gene expression in such vectors is unreliable. Defective/helper virus systems can be constructed with blocks at various stages of the replication cycle, which reliably express foreign genes but which are unable to sustain an infection. Defective infectious single cycles (DISC) viruses have been derived from both HSV types 1 and 2 with a block at the late phase of virus replication. DISC viruses kill any permissive cells they enter, but are unable to sustain infection by entering further cells ('single cycle' viruses). The most tested DISC viruses lack glycoprotein H (gH), a surface glycoprotein essential for virion infectivity and spread of virus from cell to cell by the contact route.

Such HSV vectors have been proposed for vaccination, especially against HIV, but no reports of their usefulness in prime-boost protocols have been published. In the art, there

is a strong perception that priming with naked DNA and boosting with a poxvirus such as MVA is a very effective route to prime-boost vaccination (e.g. Woodberry *et al.*, The Journal of Immunology, 2003, 170: 2599-2604).

In attempts to improve the immunogenicity of the DNA-MVA prime-boost approach, several other recombinant viruses have been evaluated in the art. These include modified fowlpoxviruses (as described in WO03/47617) and replication-deficient adenoviruses (as described in WO01/21201). Heterologous prime-boost vaccination strategies currently in clinical trials use either poxviruses, replication-deficient poxviruses or replication-deficient adenoviruses as boost vaccines, and plasmid DNA, protein formulations such as RTSS, or viruses such as BCG or replication-deficient poxviruses as primes. These conventional prime-boost strategies rely on synergistic boosting of a primed immune response to an antigen by a heterologous boosting vector encoding the same antigen. Problems with these conventional prime-boost systems include pre-existing vector immunity (to poxviruses as a result of eradication campaigns, and to adenoviruses as a result of wild-type infection) and difficulty of manufacturing replication deficient poxviruses in cell lines *in vitro*. Thus, it is a problem in the art to provide alternative vectors to replication-deficient poxviruses and adenoviruses which can be used to produce boosting of the immune response in a heterologous prime-boost system. It is a further problem to provide vectors which can be easily manufactured without the need for primary cell culture.

SUMMARY OF THE INVENTION

We have now determined that HSV vectors can be used to provide highly efficient compositions for prime-boost applications in vaccination. In particular, we have found that HSV vectors are highly efficient boosting agents when used in prime-boost applications, and are as efficient at boosting as MVA vectors. HSV vectors therefore provide a viable alternative to MVA.. Moreover, HSV DISC mutants are particularly effective as both priming and boosting compositions.

Comparisons of prime-boost regimes of the invention featuring replication-deficient herpesvirus with conventional prime-boost regimes are presented herein. Replication-

deficient herpesviruses thus provide an alternative to conventional prior art prime-boost regimes. Furthermore, replication-deficient herpesviruses in heterologous prime-boost regiments are advantageously shown to be able to produce significantly higher immune responses than DNA-MVA prime-boost.

In a first aspect, therefore, there is provided a method for vaccinating a subject against a target pathogen or disease, comprising administering at least one dose of:

(a) a first, priming, composition comprising at least one immunogenic epitope associated with the target pathogen or disease, and

(b) a second, boosting, composition comprising at least one immunogenic epitope associated with the target pathogen or disease, including at least one immunogenic epitope which is the same immunogenic epitope as the first composition,

wherein at least one of the first and second composition comprises a nucleic acid encoding said immunogenic epitope in a replication impaired HSV vector, provided that the vector used in the first composition is different to the vector used in the second composition.

In another aspect, the invention provides a method for inducing an immune response against a target pathogen or disease in a subject, comprising administering at least one dose of:

(a) a first, priming, composition comprising at least one immunogenic epitope associated with the target pathogen or disease, and

(b) a second, boosting, composition comprising at least one immunogenic epitope associated with the target pathogen or disease, including at least one immunogenic epitope which is the same immunogenic epitope as the first composition,

wherein at least one of the first and second composition comprises a nucleic acid encoding said immunogenic epitope in a replication impaired HSV vector, provided that the vector used in the first composition is different to the vector used in the second composition.

Advantageously, the second composition comprises an HSV vector. The present inventors have observed that HSV vectors provide highly efficient boosting compositions, as efficient as the MVA boosting compositions of the prior art.

Preferably, the first composition comprises an HSV vector. However, where the first and second compositions both comprise HSV vectors, the vectors are different. Advantageously, either the first composition or the second composition, but not both, comprise HSV vectors.

Preferably, the HSV vector undergoes a single round of replication in a target host cell. HSV vectors which are capable of a single round of replication in a host cell but which cannot infect other cells, for example DISC mutants, have particular advantages in prime-boost applications and have been shown to be as good as or better than the DNA-MVA "gold standard" of the prior art.

Preferably, the HSV vector has a deletion in the gH gene locus. Deletion of the gH gene in DISC mutants permits single-cycle replication in host cells, without the risk of infection of other cells. Advantageously, only the gH gene is deleted.

In a further aspect, there is provided a method for vaccinating a subject against a target pathogen or disease, comprising administering at least one dose of:

(a) a first, priming, composition comprising at least one immunogenic epitope associated with the target pathogen or disease in an HSV vector, and

(b) a second composition comprising at least one immunogenic epitope associated with the target pathogen or disease, including at least one immunogenic epitope which is the same immunogenic epitope as the first composition,

wherein the vector used in the first composition is different to the vector used in the second composition.

It has been found that HSV vectors and particularly HSV vectors which are capable of undergoing a single round of replication without further infection, such as DISC mutants

and particularly those mutants comprising a deletion in the gH gene locus only, have particularly advantageous properties as priming compositions. Such mutants prime more effectively than other HSV vectors.

Advantageously, the vector is a dH1A or dH2A DISC mutant, in which the gH gene alone has been disrupted.

The invention moreover provides for the administration of an immunomodulator in any method as set forth above.

In a further aspect, the invention relates to the use of a replication-impaired HSV vector to boost a primed immune response in an animal. Advantageously, the replication-impaired HSV vector has a deletion in the gH gene.

For example, the HSV vector is a dH1A or dH2A DISC mutant.

The invention is applicable to the vaccination of animals, especially mammals. More particularly, the invention is applicable to the vaccination of primates, including humans.

In a further aspect, the invention provides a method of boosting a pre-existing immune response in a subject comprising administering a composition comprising a replication-impaired HSV vector to said subject.

In another aspect the invention relates to a kit for inducing an immune response which comprises:

(i) a priming composition; and

(ii) a boosting composition which comprises an HSV vector.

In a still further aspect, the invention provides a vaccination kit or a kit for inducing an immune response which comprises:

(i) a priming composition which comprises an HSV vector which is dH1A or dH2A; and

(ii) a boosting composition for simultaneous, separate or sequential administration.

Moreover, the invention provides a vaccination kit which comprises:

(i) a priming composition; and

(ii) a boosting composition which comprises an HSV vector.

Preferably, the first and second compositions are capable of expressing the same antigen. Antigens are inserted into HSV vectors using inactivated gene loci. Preferably, the gH locus is used for insertion. Other gene loci may be used, including ICP4, ICP22, ICP47, ICP27, the Lat region, and combinations thereof.

Moreover, the invention provides the use of a boosting composition or a kit according to previous aspects of the invention in the manufacture of a medicament for treating and/or preventing a disease in a subject.

The methods, kits, compositions etc. according to the invention advantageously elicit cellular immune responses. Preferably, a T cell immune response is elicited in the subject.

In one embodiment, the invention provides a kit for inducing an immune response which comprises

(i) a priming composition comprising a replication-impaired viral vector

(ii) a boosting composition comprising a replication-impaired herpesvirus vector.

Preferably (i) is different from (ii). Preferably the vector of (i) is different from the vector of (ii). Preferably said kit for inducing an immune response is a vaccination kit.

In a preferred embodiment, the replication-impaired viral vector (i) and the replication-impaired herpesvirus vector (ii) each encode CD4+ and/or CD8+ T cell epitopes of an antigen.

In a preferred embodiment, the kit elicits CD4+ and/or CD8+ T cell responses to the antigen in a subject immunised with the priming composition followed by the boosting composition.

In another embodiment, the invention provides a method of inducing an immune response to one or more CD4+ and/or CD8+ T cell epitopes of a target antigen which comprises (i) immunising a subject with a priming composition comprising a replication-impaired viral vector encoding one or more of the CD4+ and/or CD8+ T cell epitopes of the target antigen
(ii) a boosting composition comprising a replication-impaired herpesvirus vector encoding at least one of the CD4+ and/or CD8+ T cell epitopes of (i). In a preferred embodiment, the vectors (i) and (ii) are different

In a preferred embodiment, the replication-impaired herpesvirus vector has a mutation by which the gH gene is inactivated or deleted.

In a preferred embodiment, the replication-impaired herpesvirus vector is selected from the group consisting of dH1A, dH1D and dH1F.

In a more preferred embodiment, the replication-impaired herpesvirus vector is dH1A.

In a preferred embodiment, the antigen is an HIV antigen or a melanoma antigen.

In a particularly preferred embodiment, the antigen is or comprises core gpn (SEQ ID NO:1).

In a preferred embodiment, the replication-impaired viral vector (i) is a replication-impaired poxvirus.

In a preferred embodiment, the replication-impaired viral vector (i) is a fowlpoxvirus.

In a particularly preferred embodiment, the replication-impaired viral vector (i) is fowlpoxvirus FP9 as described in WO03/47617.

In a preferred embodiment, the replication-impaired viral vector (i) is modified vaccinia virus Ankara (MVA).

In a preferred embodiment, the replication-impaired viral vector (i) is a replication-impaired adenoviral vector.

In a particularly preferred embodiment, the replication-impaired viral vector (i) is the replication-impaired adenoviral vector Ad5.

In another embodiment, the invention provides a kit for inducing an immune response which comprises
(i) a priming composition comprising a replication-impaired herpesvirus vector
(ii) a boosting composition comprising a replication-impaired viral vector. Preferably (i) is different from (ii). Preferably the vector of (i) is different from the vector of (ii). Preferably said kit for inducing an immune response is a vaccination kit.

In a preferred embodiment, the replication-impaired viral vector (ii) and the replication-impaired herpesvirus vector (i) each encode CD4+ and/or CD8+ T cell epitopes of an antigen.

In a preferred embodiment, the vaccination kit elicits CD4+ and/or CD8+ T cell responses to the antigen in a subject immunised with the priming composition followed by the boosting composition.

In another embodiment, the invention provides a method of inducing an immune response to one or more CD4+ and/or CD8+ T cell epitopes of a target antigen which comprises
(i) immunising a subject with a priming composition comprising a replication-impaired herpesvirus vector encoding the CD4+ and/or CD8+ T cell epitopes of the target antigen

(ii) a boosting composition comprising a replication-impaired viral vector encoding at least one of the CD4+ and/or CD8+ T cell epitopes of (i).

In a preferred embodiment, the vectors (i) and (ii) are different.

In a preferred embodiment, the replication-impaired herpesvirus vector has a mutation by which the gH gene is inactivated or deleted.

In a preferred embodiment, the replication-impaired herpesvirus vector is selected from the group consisting of dH1A, dH1D and dH1F.

In a more preferred embodiment, the replication-impaired herpesvirus vector is dH1A.

In a preferred embodiment, the antigen is an HIV antigen or a melanoma antigen.

In a particularly preferred embodiment, the antigen comprises or is core gpn (SEQ ID NO:1).

In a preferred embodiment, the replication-impaired viral vector (ii) is a replication-impaired poxvirus.

In a preferred embodiment, the replication-impaired viral vector (ii) is a fowlpoxvirus.

In a particularly preferred embodiment, the replication-impaired viral vector (ii) is fowlpoxvirus FP9 as described in WO03/47617.

In a preferred embodiment, the replication-impaired viral vector (ii) is modified vaccinia virus Ankara (MVA).

In a preferred embodiment, the replication-impaired viral vector (ii) is a replication-impaired adenoviral vector.

In a particularly preferred embodiment, the replication-impaired viral vector (ii) is the replication-impaired adenoviral vector Ad5.

Preferably the subject is mammalian, preferably human.

Preferably 'immunising with' a composition means administering said composition to a subject in such a way as to elicit an immune response. Preferably such administration is by injection. Further details are provided in the 'administration' section below.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: IFN γ ELISPOT responses elicited by dH1A.LacZ in a single immunization regimen.

Groups of female BALB/c mice ($H2^d$; n=4 mice/group) were immunised intravenously (i.v.) with $1x10^6$ plaque forming units (pfu) of dH1A.LacZ or MVA.LacZ, or intramuscularly (i.m.) with 50μg pCMV.beta DNA vaccine, as indicated. Fourteen days later, the number of IFN γ spot forming cells (SFC) per million splenocytes was determined by IFN γ ELISPOT using a β-galactosidase specific $CD8^+$ T cell epitope TPH (Table 1). Columns represent the number of SFC/million ± SD for four mice per group.

Mice immunized with pCMV-beta elicited stronger TPH-specific IFN γ responses (Table 1) than mice immunized with dH1A.LacZ ($P = 1.4x10^{-4}$). However, similar frequencies TPH-specific IFN γ secreting cells were detected in mice immunized with dH1A.LacZ and mice immunized with MVA.LacZ ($P = 0.10$).

Figure 2: IFN γ ELISPOT responses elicited by priming with HSV-vectors in a prime-boost immunization regimen.

Groups of female BALB/c mice (n=4 mice/group) were immunised i.v. with $1x10^6$ pfu of dH1A.LacZ, dH1F.LacZ or dH1D.LacZ, or i.m. with 50μg of pCMV-beta DNA vaccine as indicated. Fourteen days later, mice were boosted by i.v. administration of $1x10^6$ pfu of MVA.LacZ, as indicated. Fourteen days after boosting, the number of IFN γ SFC/million

splenocytes was determined (n=4 mice/group) by IFN γ ELISPOT using a β-galactosidase specific $CD8^+$ T cell epitope TPH. Columns represent the number of SFC/million ± SD for four mice per group.

Mice primed with dH1A.LacZ, dH1D.LacZ or dH1F.LacZ and boosted with MVA.LacZ elicited stronger TPH-specific IFN γ responses than mice immunized solely with MVA.LacZ. (P = <0.05). Similar frequencies of TPH-specific IFN γ secreting cells were detected in mice immunized with dH1A.LacZ + MVA.LacZ and mice immunized with pCMV-beta and MVA.LacZ (P= 0.07).

Figure 3: IFN γ ELISPOT responses elicited by dH1A.LacZ in homologous and heterologous prime-boost immunization regimens.

Groups of female BALB/c mice (n=4 mice/group) were immunised i.v. with $1x10^6$ pfu of dH1A.LacZ or with $1x10^6$ pfu of MVA.LacZ, or i.m. with 50μg of pCMV-beta DNA vaccine, as indicated. Fourteen days later, mice were either left untreated or boosted by i.v. administration of $1x10^6$ pfu of dH1A.LacZ or MVA.LacZ, as indicated. Fourteen days after boosting, the number of IFN γ SFC/million splenocytes was determined (n=4 mice/group) by IFN γ ELISPOT using a β-galactosidase specific $CD8^+$ T cell epitope TPH. Columns represent the number of SFC/million ± SD for four mice per group.

Mice primed with dH1A.LacZ, elicited stronger TPH-specific IFN γ responses than mice primed with pCMV-beta (P=0.02) when MVA.LacZ was given as a boost. Similar frequencies TPH-specific IFN γ secreting cells were detected in mice immunized with dH1A.LacZ and MVA.LacZ irrespective to the order of administration (P= 0.22).

Figure 4: IFN γ ELISPOT responses elicited by administration of dH1A.gpn in prime-boost immunisation regimens with other viruses and DNA plasmids

Groups of female BALB/c mice ($H2^d$; n=4 mice/group) were immunised intravenously (i.v.) with 50ug of pSG2.gpn, 1 x 10^6 plaque forming units (pfu) of dH1A.gpn, FP9.gpn

or MVA.gpn, or with 1 x 10^9 viral particles (VP) of Ad5.gpn, as indicated. Fourteen days later, mice were boosted with the recombinant viral constructs in a similar manner, as indicated. Fourteen days after boosting, the number of IFN γ SFC/million splenocytes was determined (n=4 mice/group) by IFN γ ELISPOT using using GPN-specific epitopes recognised by $CD8^+$ (AMQ) and $CD4^+$ (NPP) T cells. Columns represent the number of SFC/million ± SD for four mice per group. Groups receiving homologous immunisation regimens and those receiving heterologous immunisation regimens with or without dH1A.gpn are indicated.

Figure 5: IFN γ ELISPOT responses elicited by Ad5.gpn, dH1A.gpn and MVA.gpn following single immunisation.

Groups of female BALB/c mice ($H2^d$; n=4 mice/group) were immunised intravenously (i.v.) with 1x10^6 plaque forming units (pfu) of dH1A.gpn or MVA.gpn or with 1x10^9 viral particles (VP) of Ad5.gpn, as indicated. Seven (7) days later, the number of IFN γ spot forming cells (SFC) per million splenocytes was determined by IFN γ ELISPOT using GPN-specific $CD8^+$ (AMQ, RGP) $CD4^+$ (NPP) epitopes. Columns represent the number of SFC/million ± SD for four mice per group.

Figure 6: IFN γ ELISPOT responses elicited against a marker epitope by administration of dH1A.TRP12 in prime-boost immunisation regimens with a DNA vaccine and other viruses

Groups of female C57BL/6 mice ($H2^b$; n=4 mice/group) were immunised intravenously (i.v.) with 1 x 10^6 plaque forming units (pfu) of dH1A.TRP12, AFOX3.TRP12 or MVA.TRP12, as indicated. Fourteen days later, mice were boosted with the recombinant viral constructs in a similar manner, as indicated. Fourteen days after boosting, the number of IFN γ SFC/million splenocytes was determined (n=4 mice/group) by IFN γ ELISPOT using using a LCMV epitope (KAV) at the C-terminal end of the TRP12 fusion protein that is recognised by $CD8^+$ T cells. Dots represent the number of SFC/million per

mouse and the group mean and s.d. are indicated by bars. Groups receiving DNA vaccine – virus prime – boost and those receiving virus – virus prime – boost are indicated.

Figure 7 shows The amino acid sequence of the novel TRP12 fusion protein (SEQ ID NO:2). Key: Bold - strong stimulation against SVYDFFVWL has been reported, a nonamer variant of a previously identified TRP-2-derived B16 epitope (Bloom MB, Perry-Lalley D, Robbins PF, Li Y, el-Gamil M, Rosenberg SA, Yang JC. Identification of tyrosinase-related protein 2 as a tumor rejection antigen for the B16 melanoma. J Exp Med. 1997 Feb 3;185(3):453-9). This SVYDFFVWL variant has been shown to bind to $H2K^b$ with much higher affinity than the 8-mer minimal epitope (Schreurs MW, Eggert AA, de Boer AJ, Vissers JL, van Hall T, Offringa R, Figdor CG, Adema GJ. 2000. Dendritic cells break tolerance and induce protective immunity against a melanocyte differentiation antigen in an autologous melanoma model. Cancer Res; 60(24):6995-7001)
Underline – murine CD8+ T cell epitope from LCMV gp33
Italic – 6-His tag

DETAILED DESCRIPTION OF INVENTION

"Priming" and "boosting" compositions are defined herein as per the common useage of these terms in the art. A priming composition provides an immune response to an antigen in an organism which has not previously been vaccinated with the antigen. A boosting composition boosts an already present immune response, which is present due to priming with a priming composition or due to natural exposure to the pathogen or disease related antigen.

Antigens as used herein comprise at least one immunogenic epitope which is characteristic of a disease or pathogen and which is capable of raising an immune response. Preferably, it is a cellular immune response, such as a T cell response.

An HSV vector is a herpes simplex virus which has been incapacitated such that its replication is impaired, as defined herein; the vector also comprises at least one heterologous nucleic acid encoding an immunogenic epitope or antigen.

Viruses and viral vectors

The present invention relates to vaccination regimes using non-replicating viral vectors.

Many viral vectors are known in the art which are capable of delivering an nucleotide of interest (NOI) via infection of a target cell. Suitable recombinant viral vectors include but are not limited to adenovirus vectors, adeno-associated viral (AAV) vectors, herpes-virus vectors, retroviral vectors, lentiviral vectors, baculoviral vectors, poxviral vectors or parvovirus vectors (see Kestler *et al* 1999 Human Gene Ther 10(10):1619-32).

Examples of retroviruses include but are not limited to: murine leukemia virus (MLV), human immunodeficiency virus (HIV), equine infectious anaemia virus (EIAV), mouse mammary tumour virus (MMTV), Rous sarcoma virus (RSV), Fujinami sarcoma virus (FuSV), Moloney murine leukemia virus (Mo-MLV), FBR murine osteosarcoma virus (FBR MSV), Moloney murine sarcoma virus (Mo-MSV), Abelson murine leukemia virus (A-MLV), Avian myelocytomatosis virus-29 (MC29), and Avian erythroblastosis virus (AEV).

A detailed list of retroviruses may be found in Coffin *et al* ("Retroviruses" 1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 758-763).

Poxviruses

In a preferred embodiment the present invention provides a vaccine, priming or boosting composition which comprises a non-replicating poxvirus vector.

The family of poxviruses can be split into two subfamilies, the *Chordopoxvirinae* and the *Entomopoxviriniae*. The *Chordopoxvirinae* (poxviruses of vertebrates) include

orthopoxviruses, parapoxviruses, avipoxviruses, capripoxviruses, leporipoxviruses, suipoxviruses, molluscipoxviruses and yatapoxviruses. A review of poxviruses, their structure and organisation, biological and antigenic properties is given in Murphy et al (1995) Virus Taxonomy Springer Verlag, Vienna pp79-87. The following table (Table 2) gives some examples of species within each genus of the poxvirus family.

Table 2

Genus	Species
Orthopoxvirus	buffalopoxvirus, camelpoxvirus, cowpoxvirus, ectromelia virus, monkeypoxvirus, rabbitpoxvirus, raccoonpoxvirus, teterapoxvirus, vaccinia virus, variola virus, voleopoxvirus, skunkpoxvirus, Uasin Gishu disease virus
Parapoxvirus	bovine papular stomatis virus, orf virus, parapoxvirus of red deer in New Zealand, pseudocowpoxvirus, Auzduk disease virus, chamois contagious ecthyma, sealpoxvirus
Avipoxvirus	canarypoxvirus, fowlpoxvirus, juncopoxvirus, mynahpoxvirus, pigeonpoxvirus, psittacinepoxvirus, quailpoxvirus, sparrowpoxvirus, starlingpoxvirus, turkeypoxvirus, peacockpoxvirus and penguinpoxvirus
Capripoxvirus	goatpoxvirus, lumpy skin disease virus, sheeppoxvirus
Leporipoxvirus	hare fibroma virus, myxoma virus, rabbit fibroma virus, squirrel fibroma virus
Suipoxvirus	swinepoxvirus
Molluscipoxvirus	Molluscum contagiosum virus
Yatapoxvirus	Yaba monkey tumor virus

Kits

The present invention provides a vaccination kit which comprises an HSV vector as a priming and/or boosting composition. Where an HSV vector is not used, however, another non-replicating viral vector such as a poxvirus vector may be used.

One of the compositions may act as a "priming" composition, to be administered first, and the other composition may act as a "boosting" composition, to be administered after an appropriate time interval (such as three weeks).

The first and second non-replicating viral vectors should be sufficiently different that no significant cross-reaction occurs.

The two viral vectors may be derived from viruses belonging to different families, for example, a poxviral vector and an HSV vector.

Alternatively, the two viral vectors may be derived from the same species but be of different serotypes.

Non-replicating

The virus vectors used in the present invention should be non-replicating in the cells of the subject (for example in human cells). The term "non-replicating", "replication-deficient" or "replication-impaired" as used herein means not capable of replication to any significant extent in the majority of normal subject cells. Viruses which are non-replicating or replication-impaired may have become so naturally (i.e. they may be isolated as such from nature) or artificially e.g. by breeding *in vitro* or by genetic manipulation, for example deletion of a gene which is critical for replication. There will generally be one or a few cell types in which the viruses can be grown. A replication-impaired HSV vector, as referred to herein, is a herpes simplex virus in which at least one gene essential for virus replication has been deleted or inactivated.

Some HSV genes essential for viral replication are shown in the table below:

Glycoprotein L (UL1)	Required for production of infectious virions
Major capsid protein VP5 (UL19)	Required for capsid production
Major capsid protein VP23 (UL18)	Required for capsid production
Glycoprotein H (UL22)	Required for production of infectious virions

Major DNA binding protein (UL29)	Required for DNA replication
Glycoprotein B (UL27)	Required for infectious virus production
Major Tegument Protein (UL48) (aTIF, Vmw65)	Required for virion production
IE-175 (RSI)	Transcriptional regulator required for early and late gene expression
glycoprotein D (US6)	Required for infectious virion production
ICP27 (UL54).	Post-transcriptional regulator

Preferably the non-replicating HSV of the present invention does not comprise a deletion in or of the UL29 gene, or other genetic disruption of said gene. Preferably the non-replicating HSV of the present invention does not comprise a deletion in or of a fragment of the UL29 gene. Preferably the non-replicating HSV of the present invention comprises an intact UL29 gene, preferably a wild-type UL29 gene.

The recombinant non-replicating HSV may be derived from either HSV-1 or HSV-2.

Advantageously, the HSV vector is DISC-HSV (as described by WO92/05263 and WO9421807). Preferably, one of the specific mutants described in the table below is used. dH1A and dH2A both undergo a disabled infectious single cycle of replication whereas the others listed are completely non-replicating.

Gene deleted/inactivated	HSV-1	HSV-2
gH	dH1A	dH2A
gH +ICP4	dH1D	dH2D
gH +ICP4 + ICP22 + ICP47	dH1E	dH2E
gH +ICP4 + ICP22 + ICP47 + ICP27	dH1F	dH2F
gH +ICP4 + ICP22 + ICP47 + ICP27 + LAT region	dH1J	dH2J

Vaccines

The HSV vector of the present invention may be used in a method to treat and/or prevent a disease in a subject.

For example, the HSV vector may be employed in a vaccine which is administered to a subject for prophylactic or therapeutic purposes. The vaccine may also comprise an adjuvant (see below).

It has been found that multiple dose vaccination (for therapy or disease prevention) is often more effective than single doses. A multiple dose vaccination program involves doses of two or more different compositions.

In heterologous vaccination programs, there is a "priming" composition which is administered to the patient first and a "boosting" composition which is administered some time later. The HSV vector of the present invention may be used in a priming composition and/or a boosting composition.

A number of other compositions may be employed in heterologous vaccination programs. Other compositions include "naked DNA", non-viral vector systems and other viral vector systems.

Naked DNA (or RNA) may be linear or circular (far example, a plasmid). It may be provided in a carrier such as a liposome or in a free form.

Suitable non-viral vectors for use in the priming composition include lipid-tailed peptides known as lipopeptides, peptides fused to carrier proteins such as KLH either as fusion proteins or by chemical linkage, whole antigens with adjuvant, and other similar systems including virus-like particles (VLPs) and bacterial vectors.

If a viral vector system is used, it may be an advantage if it is derived from a different virus (i.e. not HSV) to minimise cross-reaction. The vector may be derived from an avipoxvirus , such as canary pox, or from a different genus of poxviruses. Particularly

preferred is an attenuated vaccinia vector system such as MVA or NVYAC. Other suitable viral vectors are vectors based on non-poxviruses, such as adeno virus, herpes virus and Venezuelan equine encephalitis virus (VEE). Suitable bacterial vectors include recombinant BCG and recombinant Salmonella and recombinant Listeria and Salmonella transformed with a DNA vaccine plasmid (Darji A et al 1997 Cell 91: 765-775).

Heterologous vaccination regimes

The present invention also relates generally to heterologous vaccination regimes using two different non-replicating viral vectors, one of which is HSV.

The present inventors have shown for the first time that heterologous prime-boost regimes using an HSV boosting vector is efficient in generating an immune response in a murine subject.

Triple and Multiple Regimes

The present invention also relates generally to multiply heterologous vaccination regimes, such as triply heterologous regimes, using different non-replicating viral vectors.

The invention thus provides a triple regime comprising administering to a subject three heterologous compositions. Preferably said three compositions each differ from their neighbouring composition. For example, if the first composition comprises X then the second composition will preferably differ from X. Clearly, in this embodiment, it is possible that the third composition may be similar or identical to the first composition. Preferably all three compositions are different from one another.

In one embodiment, one of the compositions may be a DNA based composition such as a DNA vaccine. Preferably at least the second and third compositions comprise non-replicating viral vectors. At least one of the compositions is an HSV vector. Particularly preferred combinations are a replication-deficient adenoviral vector, a replication-deficient pox vector, and a replication-deficient HSV vector.

T cell responses

The vaccination method or program according to the invention preferably elicits a cellular immune response, advantageously a T cell immune response, in the subject.

The nature of a T cell immune response can be characterised by virtue of the expression of cell surface markers on the cells. T cells in general can be detected by the present of TCR, CD3, CD2, CD28, CD5 or CD7 (human only). CD4+ T cells and CD8+ T cells can be distinguished by their co-receptor expression (for example, by using anti-CD4 or anti-CD8 monoclonal antibodies).

Since CD4+ T cells recognise antigens when presented by MHC class II molecules, and CD8+ recognise antigens when presented by MHC class I molecules, CD4+ and CD8+ T cells can also be distinguished on the basis of the antigen presenting cells with which they will react.

Within a particular target antigen, there may be one or more CD4+ T cell epitopes and one or more CD8+ T cell epitopes. If the particular epitope has already been characterised, this can be used to distinguish between the two subtypes of T cell, for example on the basis of specific stimulation of the T cell subset which recognises the particular epitope.

CD4+ T cells can also be subdivided on the basis of their cytokine secretion profile. The T_H1 subset characteristically secretes IL-2 and IFNγ and mediates several functions associated with cytotoxicity and local inflammatory reactions. T_H1 cells are capable of activating macrophages leading to cell mediated immunity. The T_H2 subset characteristically secretes Il-4, IL-5, IL-6 and IL-10, and is thought to have a role in stimulating B cells to proliferate and produce antibodies (humoral immunity).

T_H1 and T_H2 cells also have characteristic expression of effector molecules. T_H1 cells expressing membrane-bound TNF and T_H2 cells expressing CD40 ligand which binds to CD40 on the B cell.

CD4+ and CD8+ T cell epitopes from desired antigens may be selected for use in the present invention. CD4+ and CD8+ T cell epitopes may be selected by comparison with the literature. For example epitope maps for both HIV and HCV may be found via the the Los Alamos National Laboratories website (http://www.lanl.gov/worldview/).

Alternatively, likely epitopes may be predicted using the "ProPred" program (epitope prediction program, employing a matrix based prediction algorithm as disclosed in Sturniolo et al. Nat. Biotechnol. 17. 555-561(1999) and Singh and Raghava (2001) Bioinformatics,17(12), 1236-37).

If whole antigens are modified for use in the PrimeBoost regime it is preferred that care is taken so that any modifications of the amino acid sequence do not disrupt the amino acid sequence of known epitopes. We describe this approach in our copending international patent application PCT/GB2004/004038.

CD8+ T cell responses are preferred.

The type of T cell immune response may thus be readily determined, for example using flow cytometry.

Target antigens

The target antigen may be characteristic of the target disease. If the disease is an infectious disease, caused by an infectious pathogen, then the target antigen may be derivable from the infectious pathogen.

In general, target diseases treated by means of the present invention include infectious diseases and cancer.

Infectious diseases include: hepatitis (strains A, B and C), HIV, AIDS, malaria, influenza, Epstein-Bar virus, measles, tuberculosis, toxoplasmosis, herpes, melanomas, adenovirus infection, meningitis, bilharzia, Candida infection, Chicken pox, Chlamydia infection, Creutzfeldt-Jakob Disease, Cytomegalovirus, dengue fever, dengue haemorrhagic fever, Diphtheria (Corynebacterium diphtheriae Infection), Ebola virus infection, E. coli infection, Epstein-Barr virus, Gonorrhea (Neisseria gonorrhoeae Infection), Hansen's Disease (Leprosy), herpes simplex virus, Helicobacter pylori, Hepatitis A-E, Human Immunodeficiency Virus, Human papilloma virus, Human Parainfluenza Viruses, H.pylori infection, Legionellosis: Legionnaire's Disease (LD) and Pontiac Fever, Mumps virus infection, Pneumonia, Poliovirus infection, Rabies virus infection, Respiratory Syncytial virus, Rhinitis, Rubella, Salmonella infection, Smallpox, Streptococcus infections, Syphilis, Tetanus, Typhoid Fever (Salmonella typhi Infection) and West Nile Virus infection

Hepatitis and HIV are most preferred.

Cancer includes: Melanoma, Breast Cancer, Prostate Cancer, Bladder Cancer, Colon and Rectal Cancer, Endometrial Cancer, Kidney Cancer (Renal Cell), Leukemia, Lung Cancer, Non-Hodgkin's Lymphoma, Ovarian Cancer, Pancreatic Cancer, Skin Cancer (Non-melanoma)

Melanoma is most preferred.

Target Antigens are preferably Antigens of infectious diseases and cancer, particularly those listed above, and particularly the melanoma antigens Tyrosinase, gp100, Melan-A, MAGE-3, NY-ESO-1, TRP-1 and TRP-2)

The target antigen may be an antigen which is recognised by the immune system after infection with the disease. Alternatively the antigen may be normally "invisible" to the immune system such that the method induces a non-physiological T cell response. This may be helpful in diseases where the immune response triggered by the disease is not

effective (for example does not succeed in clearing the infection) since it may open up another line of attack.

Preferred Breast Cancer antigens are MUC-1, HER2, CEA;
Preferred Colon cancer antigens: CEA, MUC-1, MAGE-12, mutant P53;
Preferred Cervical cancer antigens: human papilloma virus proteins E6 and E7;
Preferred EBV-induced B and T cell lymphomas antigens: EBNA1 and 2, LMP 1;
Preferred renal cancer antigens: HER-2 neu, RAGE, MUC-1.

Preferred HPV antigens are viral proteins E1-8, L1 and L2
Preferred HSV antigens are viral proteins gM, gH, gK, GG, gD
Preferred HBV antigens are viral proteins small, middle and large surface antigen, core antigen, polymerase X protein.
Preferred HCV proteins are viral proteins core protein, envelope protein, NS2, NS3, NS4 and NS5 region

The antigen may be a tumour antigen, for example HER2/neu, MUC-1, MAGE-1, MAGE-3 or NY-ESO.

The antigen may be an autoantigen, for example tyrosinase or Melan-A.

In a preferred embodiment of the invention, the antigen is derivable from *M. tuberculosis*. For example, the antigen may be ESAT6 or MPT63.

In another preferred embodiment of the invention, the antigen is derivable from the malaria-associated pathogen *P. falciparum*.

The compositions of the present invention may comprise T cell epitopes from more than one antigen. For example, the composition may comprise one or more T cell epitopes from two or more antigens associated with the same disease. The two or more antigens may be derivable from the same pathogenic organism.

Alternatively, the composition may comprise epitopes from a variety of sources. For example, the ME-TRAP insert described in the examples comprises T cell epitopes from *P. falciparum*, tetanus toxoid, *M. tuberculosis* and *M. bovis*.

Target Diseases

The method of the present invention will be useful for treating and/or preventing a number of diseases, especially those which are susceptible to a T cell mediated immune response.

In particular, the method of the present invention will be useful in the treatment and/or prevention of diseases which are or are caused by chronic infections, particularly persistent, latent infections.

The compositions described herein may be employed as therapeutic or prophylactic vaccines. Whether prophylactic or therapeutic immunisation is the more appropriate will usually depend upon the nature of the disease. For example, it is anticipated that cancer will be immunised against therapeutically rather than before it has been diagnosed, while anti-malaria vaccines will preferably, though not necessarily be used as a prophylactic.

Pharmaceutical compositions/Vaccines

The present invention also relates to a pharmaceutical composition such as a vaccine, priming or boosting agent.

The pharmaceutical composition may also comprise, for example, a pharmaceutically acceptable carrier, diluent, excipient or adjuvant. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice.

In particular, a composition comprising a DNA plasmid vector may comprise granulocyte macrophage-colony stimulating factor (GM-CSF), or a plasmid encoding it, to act as an adjuvant; beneficial effects are seen using GM-CSF in polypeptide form. Adjuvants such as QS21 or SBAS2 (Stoute J A et al. 1997 N Engl J Medicine 226: 86-91) may be used with proteins, peptides or nucleic acids to enhance the induction of T cell responses.

In the pharmaceutical compositions of the present invention, the composition may also be admixed with any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), or solubilising agent(s).

The pharmaceutical composition could be for veterinary (i.e. animal) usage or for human usage. For veterinary usage, the composition may be used to treat for example mammals (especially cattle) or birds.

Preferably the subject is a mammalian subject, in particular a primate (e.g. human) or ungulate (e.g. cow) subject.

Administration

In general, a therapeutically effective intradermal or intramuscular dose of the compositions of the present invention is likely to range from 10^5-10^{10} plaque-forming units (pfu), preferably from 10^{10}-10^{11} plaque-forming units.

Typically, the physician or veterinary surgeon will determine the actual dosage which will be most suitable for an individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

Transmission of members of the *Chordopoxvirinae* may occur by aerosol (Murphy et al (1995) as above). The compositions of the present invention may also be administered by aerosol for inhalation by the subject. The compositions of the present invention may also

be conveniently administered by injection, such as intradermal and/or intramuscular injection. In addition, the compositions may be administered using a suitable device into the skin or other tissues (for example using a "gene gun" biojector or similar).

Where appropriate, the pharmaceutical compositions can be also be administered in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intracavernosally, intravenously, or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

It is also possible to administer the compositions of the present invention in sustained release formulations.

Immunomodulators

The prime and/or boost may also encode immunomodulators such as cytokines and costimulatory molecules.

Cytokines include GM-CSF (preferred), Interleukin (IL)-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, interferon (IFN)- α, IFN β, IFN γ, Macrophage Inflammatory Protein (MIP)-1α, MIP1-β, Tumour Growth Factor (TGF)-β, Tumour Necrosis Factor (TNF)-α and TNF-β.

Costimulatory Molecules include ICAM-1, LFA-3, 4-4BBL, CD59, CD40, CD70, VCAM-1 and OX-40L.

The present invention will now be described by way of example. It should be noted that these examples are not intended to be limiting in nature, but are rather intended to illustrate and to aid understanding of the invention.

Example 1

INTRODUCTION

Herpes simplex virus (HSV) replication defective mutants have been shown to be safe for use in humans and have the potential to be used as vectors for immunotherapy against cancer or against infectious diseases.

The following HSV-1-derived replication defective mutants are evaluated as antigen delivery vectors: dH1A, dH1D and dH1F. The dH1A vector has been attenuated by deletion of the gH gene. In addition to the gH gene, the gene encoding for the infected cell protein-4 (ICP4) has been deleted in the dH1D vector. The gH, ICP4, ICP22, ICP47 and ICP27 genes have been deleted in vector dH1F, rendering this virus the most attenuated of those evaluated.

These attenuated HSV vectors all express the *Escherichia coli* LacZ gene (encoding β-galactosidase) under the control of the hCMV immediate-early (IE1) promoter. The LacZ expression cassette was inserted into the essential glycoprotein H gene (gH) of HSV-1.

OBJECTIVES

The primary objective of this study was to evaluate the immunogenic potential of the attenuated HSV-1 viruses to act as priming or boosting agents in heterologous prime-boost immunisation regimens. Accordingly, this study aimed to determine the immunogenicity of dH1A, dH1D and dH1F by measuring the β-galactosidase IFN γ, IL-15, IL-18 responses in splenocytes of immunized mice using an IFN γ ELISPOT assay in the following experiments:

1) The immunogenicity of the HSV vectors in a single immunization regimen.

2) The ability of the HSV vectors to function as priming agents in combination with modified vaccinia virus Ankara expressing β-galactosidase (MVA.lacZ) in a heterologous prime-boost immunization regimen.

3) The ability of the HSV vectors to function as boosting agents in homologous and heterologous prime-boost immunization regimen with MVA.lacZ.

MATERIALS and METHODS

Constructs

The recombinant poxvirus MVA expressing beta-galactosidase (MVA.LacZ), was kept at -80°C until required. After thawing of the MVA.LacZ, virus was sonicated for exactly 1 minute prior to dilution in sterile PBS to $1x10^7$ plaque forming units (pfu)/mL. The disabled HSV constructs dH1A, dH1D and dH1F were kept at -80°C until required.

pCMV-beta plasmid DNA (1mg/ml) was made by the VDG and kept at -20°C until required.

Mice and Immunisations

Female BALB/c mice ($H2^d$; 6 – 8 weeks) were used in all experiments and kept in individually ventilated cages, in accordance with the Animals (Scientific Procedure) Act 1986 of the UK. Fifty micrograms (50ug) of the DNA plasmid, pCMV-beta, was administered by intramuscular (i.m.) injection into both *anterior tibialis* muscles (25µl per muscle). Mice were anaesthetised prior to i.m. immunisation using Hypnorm (Jensen Pharmaceutical Ltd.) and Midazolam (Hypnoval, Roche Products Ltd.).

Immunological Assays

IFN γ ELISPOT

Immune responses against the MHC class I–restricted peptide TPH recognised by $CD8^+$ T cells were determined in spleen cells by *ex vivo* IFN γ ELISPOT assay. ELISPOT assays

were performed following the standard protocol described in WO03/047617. Statistical differences between groups were determined by a single-factor ANOVA using Microsoft Excel 2002.

Table A

Peptide	Sequence	Antigen	MHC-restriction	T cell recognition
TPH	TPHPARIGL	β-galactosidase	H2-L^d	$CD8^+$

RESULTS

Experiment 1: IFN γ ELISPOT responses elicited by dH1A.LacZ in a single immunisation regimen.

Mice immunized with a single i.v. injection of $1x10^6$ pfu of dH1A.LacZ elicited detectable frequencies of TPH-specific IFN γ secreting cells (Figure 1). In contrast, a single immunization with Ad5.CMV.LacZ failed to elicit TPH-specific IFN γ responses (Figure 1). Thus, dH1A.LacZ proved to be immunogenic in mice after a single i.v. administration.

Experiment 2: IFN γ ELISPOT responses elicited by priming with HSV-vectors in a prime-boost immunization regimen.

Priming with any of the attenuated HSV constructs and boosting with MVA.LacZ induced significantly (*P<0.05*) higher frequencies of TPH-specific IFN γ responses than priming with placebo (PBS) and boosting with MVA.LacZ (Figure 2). Moreover, priming with dH1A.LacZ was found to be more potent than priming with dH1D.LacZ or dH1F.LacZ. The magnitude of the TPH-specific IFN γ response following priming with dH1A.LacZ and boosting with MVA.LacZ was comparable to that of the TPH-specific IFN γ response elicited in mice immunized with a DNA vaccine plasmid (pCMV-beta) and boosted MVA.LacZ. Thus, replication defective HSV vectors proved to be potent priming agents,

with dH1A.LacZ (in which only the gH gene has been knocked-out) proving to be the most potent priming agent.

Experiment 3: IFN γ ELISPOT responses elicited by dH1A.LacZ in heterologous and homologous prime-boost immunization regimens.

Heterologous prime-boost regimens using dH1A.LacZ were significantly more immunogenic than homologous prime-boost regimens using dH1A.LacZ (Figure 3; $p<0.05$). Furthermore, no significant difference ($P>0.05$) in the frequency of TPH-specific IFN γ secreting cells was detected in mice when dH1A.LacZ was used as a priming or boosting agent in a heterologous regimen with MVA.LacZ.

MVA.LacZ-boosted mice primed with dH1A.LacZ elicited stronger IFN γ responses than mice primed with pCMV.beta ($p<0.05$).

Thus, dH1A.LAcZ proved to be immunogenic as a priming agent, as well as a boosting agent in heterologous prime-boost regimens.

CONCLUSIONS

The HSV-1-derived replication defective mutant dH1A.lacZ, in which a single gene (gH) has been knocked-out, proved to be as immunogenic as MVA.lacZ after a single administration. This vector was also the most immunogenic priming agent when compared with dH1D.lacZ and dH1F.lacZ, which are more highly attenuated than the dH1A.lacZ vector. Heterologous prime-boost using dH1A.LacZ was more immunogenic than homologous prime-boost and this vector elicited equivalent immune responses when used as a priming agent or boosting agent in combination with MVA.lacZ. Taken together, these results indicate that the dH1A construct is an immunogenic delivery system that can be used in heterologous prime-boost immunisation regimes.

The inventors have constructed recombinant vectors which encode CD8+ T cell epitopes which are known to elicit an immune response in BALB/c mice. The epitope used has an

MHC-restriction H2-L^d and BALB/c mice are $H2^d$. Therefore the immune response elicited can be attributed to a CD8+ T cell immune response.

Figure 1 shows that dH1A is an immunogenic priming composition.

- **dH1A is at least as good as MVA for eliciting a TPH-specific immune response.**

Figure 2 shows that DISC-HSV is a good priming composition in a PrimeBoost regime.

- **There is no significant difference between the TPH-specific response elicited by dH1A/MVA and the response elicited by DNA/MVA.**

This is very surprising as DNA/MVA is considered to be an excellent immunisation regime (as shown by Schneider *et al.* Vaccine. 2001 Sep 14;19(32):4595)

DNA is highly immunogenic in mice; higher doses of plasmid DNA are needed to induce an immune response in humans. Immune responses are usually elicited in mice using 0.1-1ug of plasmid DNA administered by a gene gun and 10-100ug administered by intramuscular injection [Fynan, E. F., Webster, R. G., Fuller, D. H., Haynes, J. R., Santoro, J. C., Robinson, J. L. Proc. Natl. Acad. Sci. U. S. A., 1993, 90, 11478.]. In a clinical trial of a malaria plasmid DNA vaccine, 500-2500ug of plasmid DNA was needed to elicit a CTL response [Wang, R., Doolan, D. L., Le, T. P., Hedstrom, R. C., Coonan, K. M., Charoenvit, Y., Jones, T. R., Hobart, P., Margalith, M., Ng, J., Weiss, W. R., Sedagah, M., De Taisne, C., Norman, J. A., Hoffman, S. L. Science, 1998, 282, 476.].

Figure 1 shows that DNA is significantly better than HSV at eliciting an immune response when administered in a single shot. The skilled man would therefore assume that DNA would be significantly better than HSV at priming an immune response in a PrimeBoost regime. It is therefore highly surprising that dH1A/MVA is at least as good as DNA/MVA.

- **dH1A is significantly better than dH1D and dH1F for priming a TPH-specific immune response**

This is a highly surprising result because we would have expected the viruses with more mutations to be more immunogenic. Some of the inactivated genes are responsible for immune evasion in humans therefore their inactivation would likely make the virus more immunogenic in man.

For example ICP47 (inactivated in dH1F) is known to affect the expression of MHC class I epitopes (Neumann L, Kraas W, Uebel S, Jung G, Tampe R. The active domain of the herpes simplex virus protein ICP47: a potent inhibitor of the transporter associated with antigen processing. J Mol Biol. 1997 Oct 3;272(4):484-92.) Its presence hinders the body's ability to recognise HSV and therefore its inactivation would be expected to make the virus more immunogenic. The immune evasion mechanism is thought to proceed via transporter protein TAP (transporter associated with antigen processing).The surprising result could be explained by the fact that the interaction of ICP47 and TAP has been shown to be highly species-specific since ICP 47 has a 100 fold higher affinity for human TAP than fore murine TAP. The observed results may therefore be due to the fact that dH1A undergo a single cycle of replication whereas dH1D and dH1F cannot replicate at all.

Figure 3 shows

- **HSV heterologous PrimeBoost is significantly better than HSV homologous PrimeBoost**

  dH1A/MVA elicits a TPH-specific T cell response >10 times greater than that elicited by dH1A/dH1A – and better than DNA/MVA.

- **Both DNA/dH1A and MVA/dH1A elicit similar responses to those achieved with DNA/MVA.**

  This is very surprising as DNA/MVA is considered to be an exceptionally good immunisation regime (see Schneider *et al* above). DNA is shown by Figure 1 to be significantly better than MVA when used in a single shot. However HSV manages to boost the DNA and MVA responses to a level only usually seen with the highly successful DNA/MVA regime.

Example 2

In Example 1, the capacity of replication impaired vectors dH1A, dH1D and dH1F to elicit immune responses against a model antigen (β-galactosidase) was demonstrated. Although each of the vectors elicited an immune response against β-galactosidase, the dH1A vector proved to be most potent in eliciting an immune response against this model antigen. This construct proved equally as potent in eliciting an immune response against β-galactosidase when used as either a priming agent or boosting agent in a heterologous prime-boost immunisation regimen with an MVA vector encoding the same antigen. Therefore, the dH1A construct shows great potential as a vector for eliciting immune responses against disease-specific recombinant antigens.

The immunogenic potential of the dH1A virus was further evaluated. Accordingly, dH1A virus recombinant viruses containing several disease-specific viral and cancer antigens were constructed. The immunogenicity of novel PrimeBoost immunisation regimens using these viruses in combination with other recombinant viral vectors was determined by measuring the level of antigen-specific T cells capable of secreting IFN-γ in splenocytes of immunised mice using an IFN-γ ELISPOT assay.

The construct known as GPN, described in WO2005/030964A1, is a synthetic fusion antigen comprising amino acid sequences of HIV-1 Clade B gag, pol and nef genes which are thought to be key targets for eliciting $CD8^+$ T cell immune responses against this virus. The amino acid sequences have been scrambled, so that the structure and activity of the HIV proteins has been destroyed but substantially all of the T cell epitopes of the HIV proteins are still present in the gpn sequence.

Heterologous immunisation regimens using MVA.gpn and FP9.gpn have been extensively tested in BALB/c mice as described in WO2005/030964A1. WO2005/030964A1 also describes heterologous regimens using these vectors and the Ad5.gpn vector evaluated in rhesus macaque monkeys. Therefore, heterologous prime-boost regimens using dH1A.gpn were compared with those using the other GPN-encoding vectors

Vector Construction

The GPN fusion protein is a synthetic fusion antigen comprising amino acid sequences of the HIV-1 Clade B Gag, Pol and Nef proteins, and has been described previously (WO2005/030964A1). Construction of MVA.gpn and FP9.gpn poxvirus vectors encoding the GPN antigen have also been described (WO2005/030964A1). The poxviruses vectors were stored at -80°C as concentrated stocks at 1 x 10^8 pfu/ml in phosphate buffered saline (PBS).

The GPN fusion protein was inserted into the gH locus of dH1A DISC HSV by homologous recombination. In brief, the gene encoding the GPN antigen was inserted into shuttle vector pGTK66 between regions flanking the gH coding region to from pGTK66.gpnR. Expression of the GPN antigen in this vector was driven by the CMV promoter with a BGH polyA termination sequence. Recombinant dHIA.gpn was produced by homologous recombination of dH1A.K24 (a DISC HSV in which gfp is inserted in the gH locus) with pGTK66.gpnR. Two independent plaques were purified such that the recombinant virus was free of parental virus prior to the preparation of bulk stocks. The composition of the resultant recombinant viruses was verified by PCR and the expression of the GPN protein confirmed by Western blotting using an antibody specific for HIV p24. Stocks of the virus were stored at -80°C at a concentration of 5.1 x 10^7 pfu/ml in PBS.

An attenuated Adenovirus 5 vector expressing the GPN antigen under the hCMV IE1 promoter was constructed using the AdEasy™ Adenoviral vector system. The recombinant virus (QBiogene, manufacturing date 17 Mar 2004) was supplied and stored as a frozen (-80°C) suspension (1.0 x 10^{11} particles/ml) in 20mM Tris, 25mM NaCl, 2.5% glycerol, pH 8.0.

Recombinant poxvirus and HSV vectors were prepared for administration to mice by dilution to 1 x 10^7 pfu/ml in sterile phosphate buffered saline solution (PBS). The Ad5.gpn vector was diluted to 1.0 x 10^{10} particles/ml in PBS on the day of immunisation.

Mice and Immunisations

Female BALB/c mice ($H2^d$; 6 – 8 weeks) were used in all experiments and kept in individually ventilated cages, in accordance with the Animals (Scientific Procedure) Act 1986 of the UK. Recombinant HSV and poxviruses were administered intravenously (i.v.) at a dose of 1×10^6 pfu and adenovirus recombinants were administered via the same route at a dose of $1x10^9$ particles. For DNA vaccination, fifty micrograms (50ug) of a DNA vaccine plasmid pSG2.gpn which expresses gpn under the CMV promoter was administered by intramuscular (i.m.) injection into both *anterior tibialis* muscles (25µl of 1 mg/mL solution per muscle). Mice were anaesthetised prior to i.m. immunisation using Hypnorm (Jensen Pharmaceutical Ltd.) and Midazolam (Hypnoval, Roche Products Ltd.).

Immunological Assays

IFN γ ELISPOT

Immune responses against MHC class I or class II –restricted peptides (Table B) recognised by $CD8^+$ and $CD4^+$ T cells, respectively, were determined in spleen cells by *ex vivo* IFN γ ELISPOT assay. ELISPOT assays were performed using the standard protocol described in WO03/047617.

Table B

Peptide	Sequence	Disease Model	Antigen	MHC-restriction	T cell recognition
AMQ	AMQMLKETI	HIV	Gag	H-$2K^d$	$CD8^+$
RGP	RGPGRAFVTI	HIV	Env	H-$2D^d$/H-$2IA^d$	$CD8^+$/$CD4^+$
NPP	NPPIPVGEIYKR WIILGLNK	HIV	Gag	-	$CD4^+$
KAV	KAVYNFATCGI	LCMV	GP	H-$2D^b$/K^b	$CD8^+$
SVY	SVYDFFVWL	B16 melanoma	TRP2	H-$2K^b$	$CD8^+$

Single Dose Treatments

Constructs and mice were the same as described, with the difference that only a single immunisation of each GPN-expressing vector was performed.

IFN γ ELISPOT responses elicited against the GPN fusion protein by a single dose of dH1A.gpn

Single doses of dH1A.gpn, Ad5.gpn or MVA.gpn administered i.v. to BALB/c mice elicited IFN γ immune responses against GPN-specific T cell epitopes (**Figure 5**); indicating that each of these constructs was effective in eliciting an immune response against the novel GPN fusion protein. Interestingly, the Ad5.gpn construct elicited the most potent T cell immune response against GPN, exhibiting an equivalent magnitude of response to both $CD8^+$ T cell epitopes tested, as well as a showing a response to the $CD4^+$ T cell epitope. In contrast, dH1A.gpn and MVA.gpn elicited IFN γ immune responses against the $CD8^+$ T cell epitopes only, and the response directed against the RGP model epitope was significantly greater than that elicited against the GPN-derived AMQ epitope. Notably, the magnitude of the T cell response elicited by the dH1A.gpn construct was significantly lower than that elicited by MVA.gpn, indicating that the dH1A.gpn was the least immunogenic of the 3 constructs tested after the administration of a single dose.

IFN γ ELISPOT responses elicited by dH1A.gpn in heterologous and homologous prime-boost immunisation regimens.

Heterologous prime-boost regimens using dH1A.gpn elicited higher frequencies of IFNγ-secreting GPN-specific $CD8^+$ and $CD4^+$ T cell responses than homologous prime-boost immunisation regimens using this vector, FP9.gpn or MVA.gpn (**Figure 4**). As summarised in Tables 3 and 4 below, he magnitude of the immune response elicited against the $CD8^+$ and CD4+ T cell epitopes was similar whether dH1A.gpn was administered as a heterologous priming or boosting agent. Therefore, dH1A.gpn appeared to be equally potent in eliciting immune responses when used as priming or boosting agent in combination with FP9.gpn or MVA.gpn. Notably, the magnitude of the T cell immune responses elicited by regimens using dH1A were not significantly different in magnitude to the response elicited by MVA.gpn prime FP9.gpn boost regimen, which is the most preferred regimen described in WO2005/030964A1.

Adenovirus Ad5.gpn had an active virus:viral particle ratio of 1:20 as measured by $TCID_{50}$ titration. That is, the standard dose of 10^9 viral particles of adenovirus used in this example is considered equivalent to 5×10^7 plaque forming units (pfu) of active virus. Therefore, in the experiments comparing adenovirus with DISC-HSV as a prime and boost, heterologous prime-boost with 1×10^6 pfu of DISC-HSV according to the present invention was still able to produce immune responses that were comparable with the case when 5×10^7 pfu of adenovirus was substituted for DISC-HSV, as shown in Tables 3 and 4 and Figure 4. The relatively high responses to adenovirus seen in Figure 4 can be considered as a dose-response effect, rather than showing any superiority of adenovirus over DISC-HSV in inducing T cell immune responses.

Table 3: Mean responses ± standard deviation to the CD8+ T cell epitope after various combinations of priming and boosting agents (spot forming cells per million splenocytes). Values in bold represent heterologous prime-boost regimen with a replication-impaired herpesvirus as a prime or a boost, according to the present invention.

Prime / Boost	pSG12.gpn (plasmid)	Ad5.gpn	MVA.gpn	FP9.gpn	dH1A.gpn
Ad5.gpn	1946 ± 442	2265 ±600	3296 ±523	3296 ±976	2062 ±770
MVA.gpn	3757 ±470	5284 ±538	1435 ±209	2306 ±472	3335 ±270
FP9.gpn	1095 ±271	4081 ±1126	4726 ±1606	146 ±89	3823 ±1553
dH1A.gpn	907 ±427	3139 ±683	2548 ±261	2964 ±632	158 ±39

Table 4: Mean responses ± standard deviation to the CD4+ T cell epitope after various combinations of priming and boosting agents. (spot forming cells per million PBMC). Values in bold represent heterologous prime-boost regimen with a replication-impaired herpesvirus as a prime or a boost, according to the present invention.

Prime / Boost	pSG12.gpn (plasmid)	Ad5.gpn	MVA.gpn	FP9.gpn	dH1A.gpn
Ad5.gpn	635 ±146	467 ±157	1319 ±359	1142 ±122	736 ±195
MVA.gpn	316 ±71	1068 ±206	239 ±35	505 ±161	635 ±30
FP9.gpn	176 ±85	794 ±258	1025 ±606	26 ±36	966 ±424
dH1A.gpn	215 ±43	978 ±273	715 ±88	823 ±234	70 ±13

Example 3: IFN γ ELISPOT responses elicited by dH1A.TRP12 in heterologous and homologous prime-boost immunisation regimens.

Vector Construction

A synthetic fusion protein (TRP12) based on the the murine Tyrosinase related protein (TRP) 1 and 2 was designed for use as therapeutic antigens in the treatment of melanoma in a mouse model. TRP1 (Jackson, I. J., D. M. Chambers, et al. (1991). "The tyrosinase-related protein-1 gene has a structure and promoter sequence very different from tyrosinase." Nucleic Acids Res 19(14): 3799-804) and TRP2 (Jackson, I. J., D. M. Chambers, et al. (1992). "A second tyrosinase-related protein, TRP-2, maps to and is mutated at the mouse slaty locus." Embo J 11(2): 527-35) are weak melanoma tumour-specific antigens expressed in murine and human cells.

Immunity against these antigens has been associated with protection from prophylactic challenge with B16 mealoma in murine models. The gene sequence encoding the novel TRP12 protein was synthesised using overlapping oligonucleotides. The codon usage of this gene was optimised for expression by adapting to that of highly expressed mouse genes. The synthetic gene was also designed to include a flexible linker, composed of three series of four glycine residues separated by one serine, was added between the TRP-1 and the TRP-2, to allow correct folding of the proteins' secondary structure. A sequence encoding an epitope recognized by murine $CD8^+$ T cells specific for the lymphocytic choriomeningitis virus (LCMV) gp33 protein (KAVYNFATCGl) was added at the C-terminus of the TRP-12 to serve as a model epitope for monitoring immune responses in C57BL/6 mice. An additional six histidine residues (6HIS tag) were added at the TRP12 carboxy-terminus to allow the detection of TRP12 recombinant vectors in antibody-based immunoassays. The resulting amino acid sequence of the TRP12 protein is shown in Figure 7.

The gene encoding the TRP12 fusion protein was inserted into the TK locus of the MVA viral vector by homologous recombination to make vector MVATRP12. The construction of MVA.TRP12 was identical to that described for the construction of MVA.gpn (Example 2), except the gene encoding TRP12 was inserted into shuttle vector pOPK26,

which encodes a gpt-GFP fusion protein as a marker for *in vitro* selection of recombinant virus in place of the B-galactosidase protein.

The gene encoding the novel TRP12 fusion protein was also inserted into "Deletion 13" of the FP9 genome by homologous recombination to make vector AFOX3.TRP12. The method used was identical that used to make FP9.gpn (Example 2), except that the TRP12 gene was inserted into shuttle vector pFd1326, which targets "Deletion 13" in the viral genome and encodes a gpt-GFP fusion protein as a marker for *in vitro* selection of recombinant viruses.

A DISC HSV (dH1A) virus expressing the TRP12 fusion protein was also constructed by homologous recombination. The method was identical to that used to construct dH1A.gpn (Example 2), except the gene encoding TRP12 was inserted into the pGTK66 shuttle vector in place of the *gpn* gene.

Storage and handling of the poxvirus and HSV vectors encoding TRP12 was as described in Example 2. The DNA vaccine vector was amplified and stored at -20°C at 1 mg/ml in PBS ready for administration to mice.

Mice and Immunisations

Female C57BL/6 mice ($H2^b$; 6 – 8 weeks) were used in all experiments and kept in individually ventilated cages, in accordance with the Animals (Scientific Procedure) Act 1986 of the UK. Recombinant HSV and poxviruses were administered intravenously (i.v.) at a dose of 1×10^6 pfu. Mice were anaesthetised prior to i.m. immunisation using Hypnorm (Jensen Pharmaceutical Ltd.) and Midazolam (Hypnoval, Roche Products Ltd.).

Immunological Assays

IFN γ ELISPOT

Immune responses against the LCMV epitope (KAV; Table B) or TRP-2-specific epitope (SVY; Table B) recognised by $CD8^+$ and $CD4^+$ T cells, respectively, were determined in spleen cells by *ex vivo* IFN γ ELISPOT assay. ELISPOT assays were performed using the standard protocol described in WO03/047617.

The immunogenicity of the dH1A construct expressing the TRP12 fusion antigen was tested in mice in heterologous prime-boost immunisation regimens in combination with a fowlpox FP9-based vector (AFOX3.TRP12) and an MVA-based vector (MVA.TRP12) expressing the same antigen. Potent immune responses were elicited against the marker epitope when the viruses were administered in heterologous virus-virus prime-boost regimens (Figure 6). Although these regimens elicited immune responses that were not significantly different from each other, it is notable that priming with dH1A.TRP12 or MVA.TRP12 and boosting with AFOX3.TRP12 elicited responses of the greatest magnitude and consistency.

The TRP2 antigen encodes an H-2^b restricted epitope (SVY), which is recognised by $CD8^+$ T cells (Bloom MB, Perry-Lalley D, Robbins PF, Li Y, el-Gamil M, Rosenberg SA, Yang JC. Identification of tyrosinase-related protein 2 as a tumor rejection antigen for the B16 melanoma. J Exp Med. 1997 Feb 3;185(3):453-9.). T cell responses against this epitope have been shown to correlate with protection against B16 tumour challenge in C57BL/6 mice; however this self epitope is known to be subject to tolerance (Schreurs MW, Eggert AA, de Boer AJ, Vissers JL, van Hall T, Offringa R, Figdor CG, Adema GJ. 2000. Dendritic cells break tolerance and induce protective immunity against a melanocyte differentiation antigen in an autologous melanoma model. Cancer Res; 60(24):6995-7001). IFN γ T cell responses elicited against this epitope in heterologous prime-boost immunisation regimens were modest.

Since the marker epitopes of the TRP12 fusion antigen demonstrated good immune responses after prime-boost immunisations with non-replicating viral vectors including dH1A, this suggests that although an epitope-specific immune response to SVY was modest, the construct and the delivery systems were immunogenic and that immune responses may have been generated to one or more other epitopes in the TRP12 fusion antigen.

Example 4:

The effect of heterologous prime-boost regiments using TRP12 expressing constructs on metastatic melanoma is evaluated *in vivo* using C57BL/6 mice.

Naïve C57BL/6 mice either receive prime-boost immunotherapy using constructs expressing the mouse melanoma fusion protein TRP12 or are left untreated prior to challenge by intra-venous administration of $1x10^5$ malignant B16 cells into the lateral tail vein. B16 mouse melanoma cells express tyrosinase related proteins 1 and 2 and therefore constitute a relevant model for evaluating the protective effect of the TRP12 expressing constructs in heterologous prime-boost.

Intra-venous injection of $1x10^5$ malignant B16 cells into the lateral tail vein commonly results in tumour colonization of the lungs within 25 days. Challenged mice rapidly lose total body weight, whereas the lungs increase in weight due to the local inflammatory reaction elicited. Following injection, mice are monitored regularly by determining the Body Weight Change (BWC) during the course of the experiment. Mice are sacrificed 25 days after B16 tumour cell inoculation. Autopsy studies are conducted; lungs are dissected, weighed and the pulmonary tumours quantified by enumeration and determination of size of individual tumours.

CONCLUSIONS

The HSV-1-derived replication defective mutant dH1A, in which a single gene (gH) has been knocked-out, proved to be a suitable vector for eliciting T cell immune responses against the HIV and murine melanoma- derived novel fusion proteins. Thus, it is demonstrated that this vector finds application as, or in use in, immunotherapeutic products against HIV and cancer.

The GPN fusion protein is composed of sequences from the Gag, Pol and Nef genes of HIV-1 Clade B, which are thought to be key targets for eliciting $CD8^+$ T cell immune responses against this virus. Therefore, this is the first observation that the dH1A virus

can be used as a vector to elicit disease-specific immune responses against a recombinant viral antigen. Heterologous prime-boost using dH1A.gpn elicited more potent T cell immune responses than homologous prime-boost immunisation with this vector or with FP9.gpn and MVA.gpn. As observed previously when dH1A was used as a vector for the β-galactosidase model antigen, this vector elicited equivalent immune responses when used as a priming agent or boosting agent in combination with FP9.gpn and MVA.gpn.

The TRP12 fusion protein is composed of sequences from the TRP1 and TRP2 murine melanoma antigens, which are though to be targets for protective immunity. Prime-boost immunisation regimens using dH1A.TRP12 induced substantial $CD8^+$ T cell immune responses against a marker epitope at the C-terminal of this fusion protein.
Overall, this study indicates that the dH1A construct is an immunogenic delivery system that can be used to elicit T cell immune responses against disease-specific viral and cancer antigens. This shows that replication-impaired herpesviruses can serve equally as well as priming or boosting agents in heterologous prime-boost immunisation regimes with other recombinant viruses.

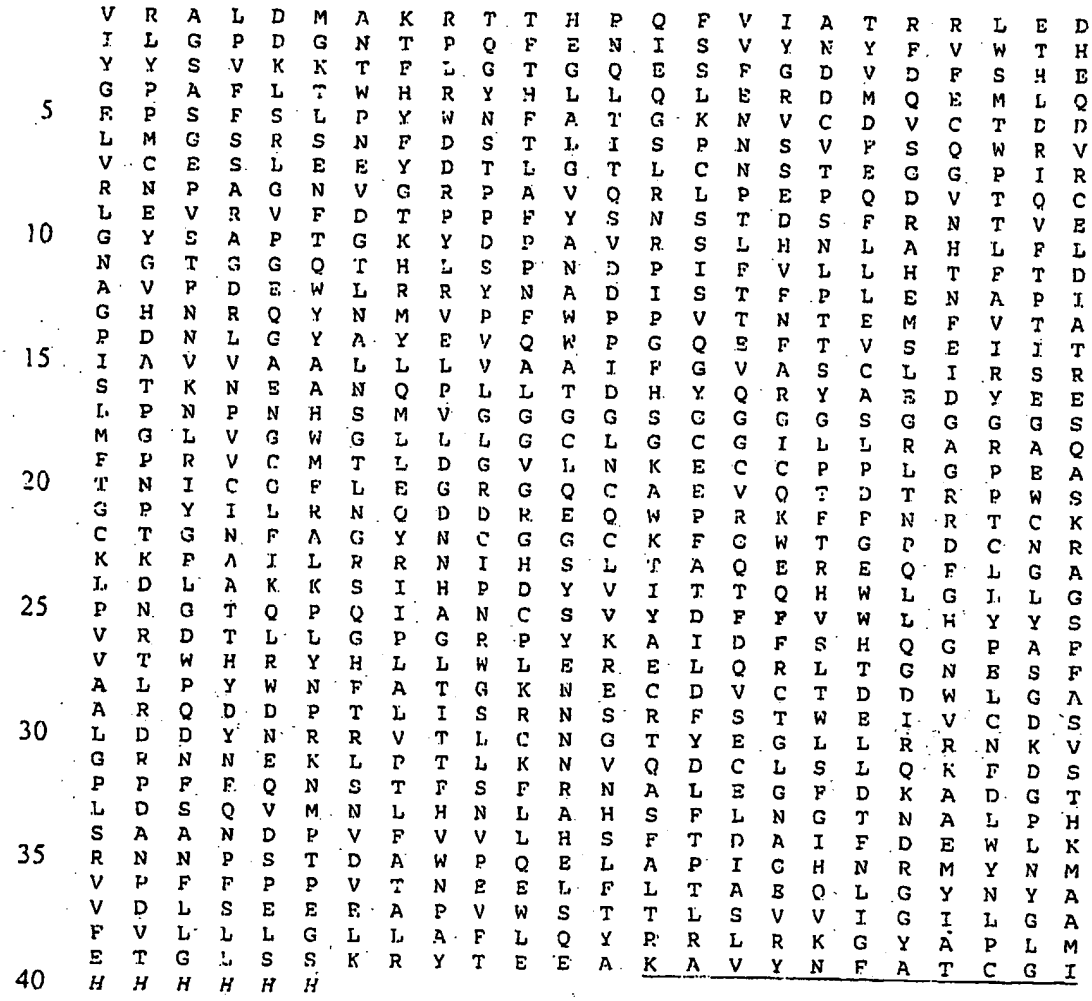
All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the present invention will be apparent to those skilled in the art without departing from the scope of the present invention. Although the present invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biochemistry and biotechnology or related fields are intended to be within the scope of the following claims.

SEQ ID NO: 1 Artificial amino acid sequence - core GPN: HIV gag/pol/nef deactivated but preserving epitopes

MAPIVQNLQGQMVHQAISPRTLNAWVKVVEEKAFSP
EVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLK
ETINEEAAEWDRLHPVHAGPIAPGQMREPRGSDIAG
TTSTLQEQIGWMTNNPPIPVGEIYKRWIILGLNKIV
RMYSPTSILDIRQGPKEPFRDYVDRFYKTLRAEQAS
QEVKNWMTETLLVQNANPDCKTILKALGPAATLEEM
MTACQGVGGPGHKARVLMAARASVLSGGELDRWEKI
RLRPGGKKKYKLKHIVWASRELERFAVNPGLLETSE
GCRQILGQLQPSLQTGSEELRSLYNTVATLYCVHQR
IEVKDTKEALEKIEEEQNKSKKKAQQAAADTGNSSQ
VSQNYTPDKKHQKEPPFLWMGYELHPDKWTVQPIVL
PEKDSWTVNDIQKLVGKLNWASQIYAGIKVKQLCKL
LRGTKALTEVIPLTEEAELELAENREILKEPVHGVY
YDPSKDLIAEIQKQGQGQWTYQIYQEPFKNLKTGKY
ARMRGAHTNDVKQLTEAVQKIATESIVIWGKTPKFK
LPIQKETWEAWWTEYWQATWIPEWEFVNTPPLVKLW
YQLEKEPIVGAETFPISPIETVPVKLKPGMDGPKVK
QWPLTEEKIKALVEICTEMEKEGKISKIGPENPYNT
PVFAIKKKDSTKWRKLVDFRELNKRTQDFWEVQLGI
PHPAGLKKKKSVTVLDVGDAYFSVPLDKDFRKYTAF
TIPSINNETPGIRYQYNVLPQGWKGSPAIFQSSMTK
ILEPFRKQNPDIVIYQYMDDLYVGSDLEIGQHRTKI
EELRQHLLRWGFTTPDKKHQKEPPFLVWKFDSRLAF
HHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSL
LHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPL
TFGWCFKLVPVEPEKVEEWQNYTPGPGIRYQKRQDI
LDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTY
KAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAAD
CAWLEAQEEEEVGFPVRPQVPLRNTAANNADCAWLA
DGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVG
AMGGKWSKRSVVGWPTVRERMRRAEPA

SEQ ID NO:2 The amino acid sequence of the novel TRP12 fusion protein

MKSYNVLPLAYISLFLMLFYQVWA
QFPRECANIEALRRGVCCPDLLPS
SGPGTDPCGSSSGRGRCVAVIADS
RPHSRHYPHDGKDDRENFSFFN
RTCQCNDNFSGHNCGTCRPGWRGA
ACNQKILTVRRNLLDLSPEEKSHF


V I Y G E L V R L G N A G P I S L M F T G C K L P V V A A L G P L S R V V F E H
R L Y P P M C N E Y G V H D A T P G P N P T K D N R T L R D R P D A N P D V T H
A G S A S G E P V S T P N N V K N L R I Y G P L G D W P Q D N F S A N F L L G H
L P V F F S S A R A G D R L V N P V V C I N A T T H Y D Y N E Q N P F S L L H
D D K L S R L G V P G E Q G A E N G C C L F I K Q L R W D N E Q V D S P E L S H
M G K T L S E N F T Q W Y Y A A H W M F R A L K P L Y N P R K N M P T P E G S H
A N T W P N E V D G T L N A L N S G T L N G R S Q G H F T R L S N V D V E L K
K T F H Y F Y G T K H R M Y L Q M L L E Q Y R I I P L A L V P T L F A T A L R
R P L R W D D R P Y L R V E L P V L D G D N N H A G L T I T T F H V W N P A Y
T Q G Y N S T P P D S Y P V V L G L G R D C I P N R W G S L L S N V P E V F T
T F T H F T L A F P P N F Q A L G G V G R G H D C P L K R C K F L L Q E W L E
H E G L A L G V Y A N A W W A T G C L Q E G S Y S Y E N N N N R A H E L S Q E
P N Q L T I T Q S V D D P P I D G L N C Q C L V V K R E S G V N H S L F T Y A
Q I E Q G S L R N R P I P G F H S G K A W K T I Y A E C R T Q A S F A L T R K
F S S L K P C L S S I S V Q G Y G C E E P A T D I L D F Y D L F T P T L R A
V V F E N N P T L F T T E V Q G G C V R C Q T F D Q V S E C E L D I A S L V
I Y G R V S S E D H V F N F A R G I C Q K W E Q F F R C T G L G N A C E V R Y
A N D D C V T P S N L P T T S Y G L P T F T R H V S L T W L S F G I H Q V K N
T Y V M D P E Q F L L L E V C A S L P D F G E W W H T D E L L D T F N L I G F
R F D Q V S G D R A H E M S L E G R L T N P Q L L Q G D I R Q K N D R G G Y A T
R V F E C Q G V N H T N F E I D G A G R R R D F G H G N W V R K A A E M Y I A C
L W S M T W P T T L F A V I R Y G R P P T C L L Y P E L C N F D L W Y N L P C
E T H L D R I Q V F T P T I S E G A E W C N G L Y A S G D K D G P L N Y G L G
D H E Q D V R C E L D I A T R E S Q A S K R A G S F F A S V S T H K M A A M I

Claims

1. A method for vaccinating a subject against a target pathogen or disease, comprising administering at least one dose of:

(a) a first, priming, composition comprising at least one immunogenic epitope associated with the target pathogen or disease, and

(b) a second, boosting, composition comprising at least one immunogenic epitope associated with the target pathogen or disease, including at least one immunogenic epitope which is the same immunogenic epitope as the first composition,

wherein at least one of the first and second composition comprises a nucleic acid encoding said immunogenic epitope in a replication impaired HSV vector, provided that the vector used in the first composition is different to the vector used in the second composition.

2. A method according to claim 1, wherein second composition comprises an HSV vector.

3. A method according to claim 1 or claim 2, wherein the first composition comprises an HSV vector.

4. A method according to any preceding claim, wherein the HSV vector undergoes a single round of replication in a target host cell.

5. A method according to claim 4, wherein the HSV vector has a deletion in the gH gene locus.

6. A method for vaccinating a subject against a target pathogen or disease, comprising administering at least one dose of:

(a) a first, priming, composition comprising at least one immunogenic epitope associated with the target pathogen or disease in an HSV vector, and

(b) a second composition comprising at least one immunogenic epitope associated with the target pathogen or disease, including at least one immunogenic epitope which is the same immunogenic epitope as the first composition,

wherein the vector used in the first composition is different to the vector used in the second composition.

7. A method according to claim 6, wherein the HSV vector is a DISC mutant.

8. A method according to claim 7, wherein the HSV vector undergoes a single round of replication in a target host cell.

9. A method according to claim 8, wherein the DISC mutant comprises a deletion in the gH gene locus.

10. A method according to any preceding claim, further comprising the administration of an immunomodulator.

11. Use of a replication-impaired HSV vector to boost a primed immune response in an animal.

12. Use of a replication-impaired HSV vector having a deletion in the gH gene to prime an immune response in an animal.

13. Use according to claim 12, wherein the HSV vector is a dH1A or dH2A DISC mutant.

14. Use according to any one of claims 11 to 13 wherein said animal is a mammal.

15. Use according to claim 14 wherein said mammal is a primate.

16. Use according to claim 15 wherein said primate is a human.

17. A method of boosting a pre-existing immune response in a subject comprising administering a composition comprising a replication-impaired HSV vector to said subject.

18. A vaccination kit which comprises:

(i) a priming composition which comprises an HSV vector which is dH1A or dH2A; and

(ii) a boosting composition for simultaneous, separate or sequential administration.

19. A vaccination kit which comprises:

(i) a priming composition; and

(ii) a boosting composition which comprises an HSV vector.

20. A kit for inducing an immune response which comprises:

(i) a priming composition; and

(ii) a boosting composition which comprises an HSV vector.

21. A kit according to any of claims 18 to 20, wherein the first and second compositions are capable of expressing the same antigen.

22. The use of a kit according to any of claims 18 to 21 in the manufacture of a medicament for treating and/or preventing a disease in a subject.

23. A method for inducing an immune response against a target pathogen or disease in a subject, comprising administering at least one dose of:

(a) a first, priming, composition comprising at least one immunogenic epitope associated with the target pathogen or disease, and

(b) a second, boosting, composition comprising at least one immunogenic epitope associated with the target pathogen or disease, including at least one immunogenic epitope which is the same immunogenic epitope as the first composition,

wherein at least one of the first and second composition comprises a nucleic acid encoding said immunogenic epitope in a replication impaired HSV vector, provided that the vector used in the first composition is different to the vector used in the second composition.

24. A method, composition, kit or use according to any preceding claim, which elicits a T cell immune response in the subject.

Figure 1

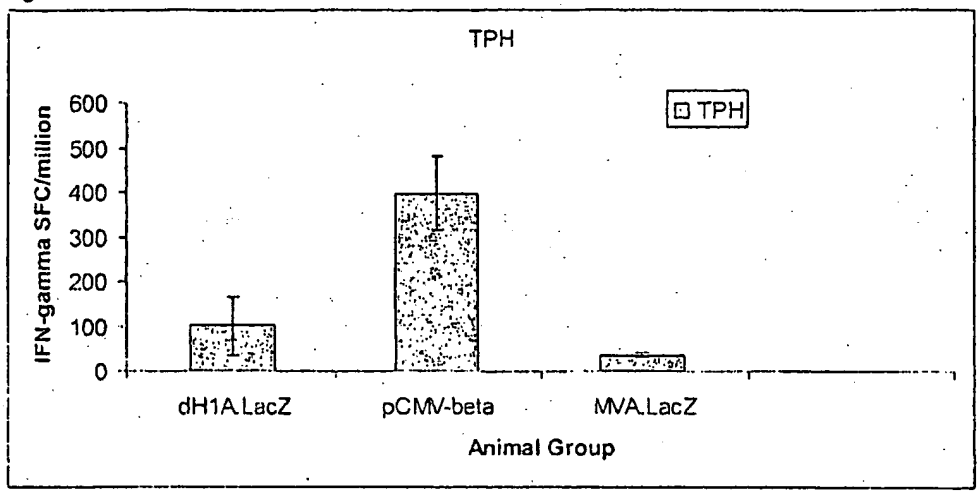


Figure 2

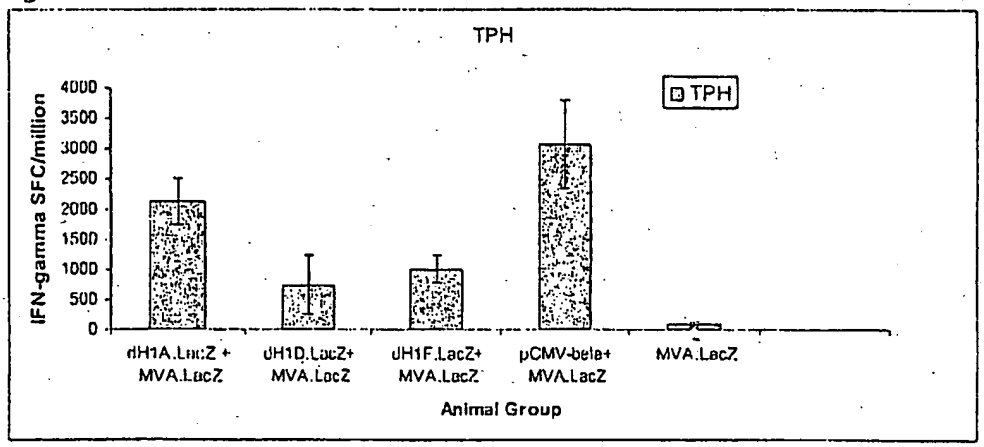


Figure 3

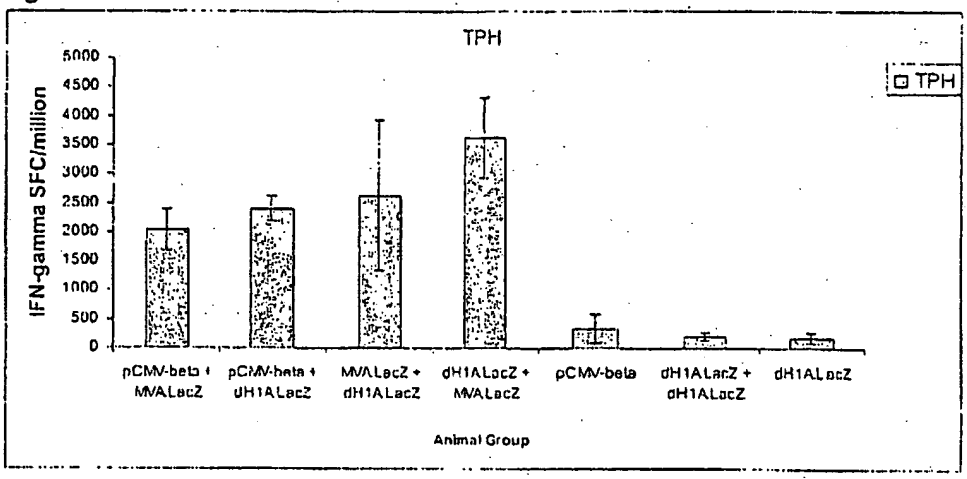


Figure 4

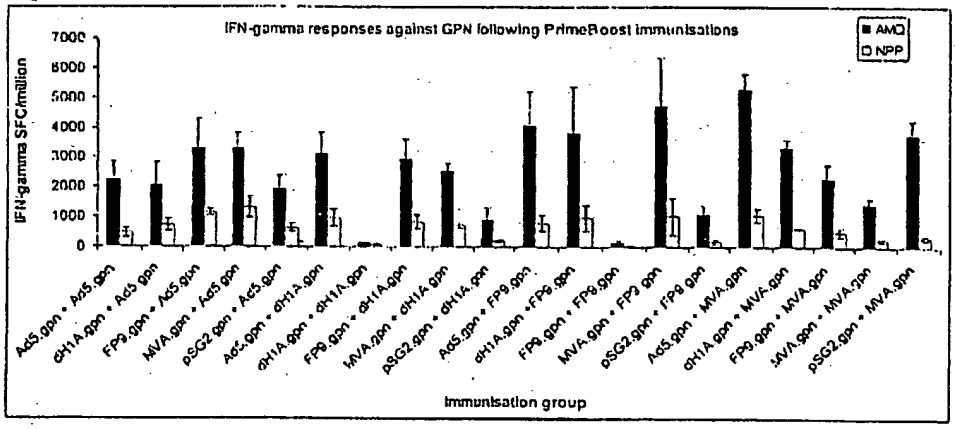


Figure 5

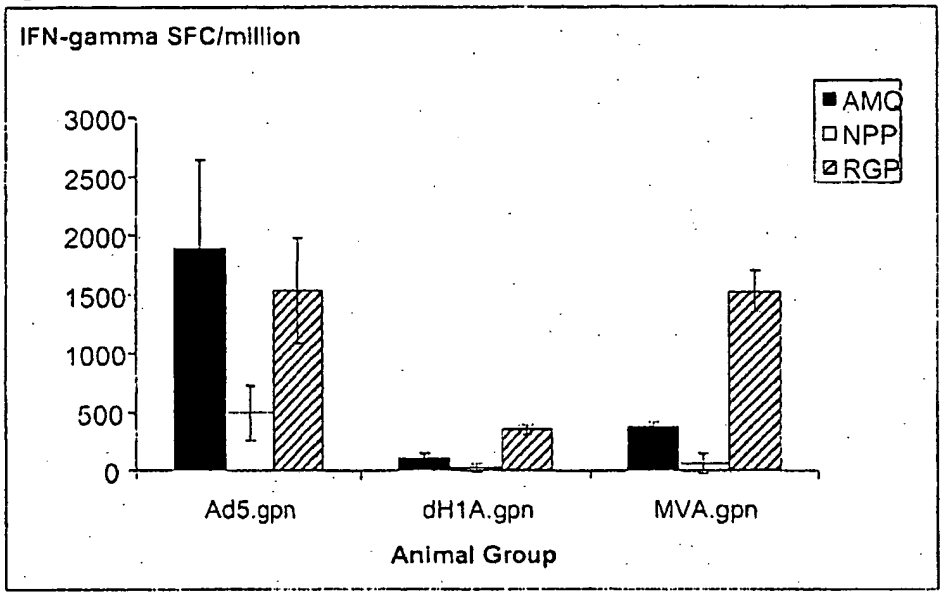


Figure 6

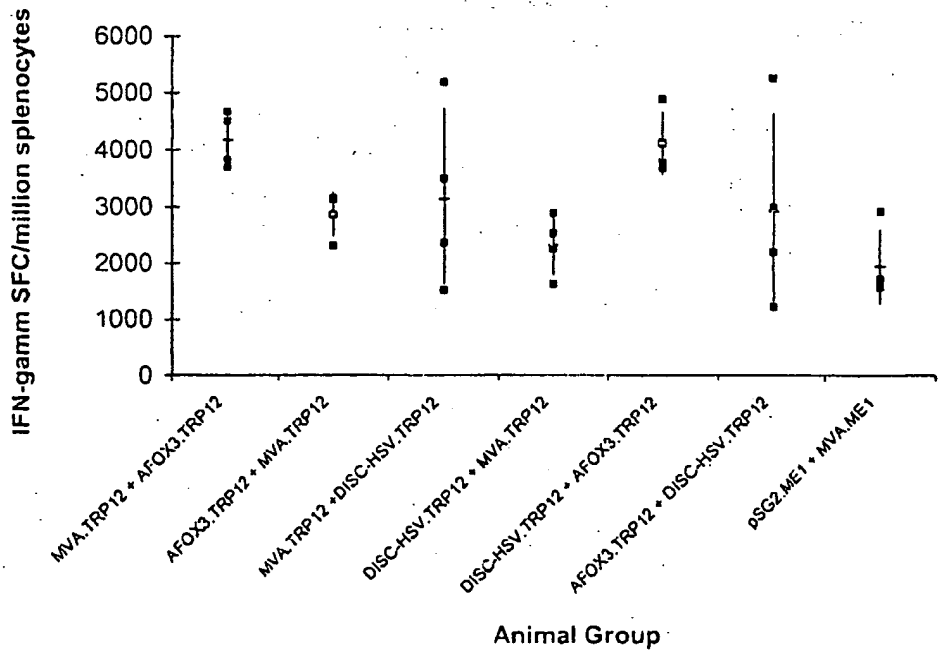


Figure 7: The amino acid sequence of the novel TRP12 fusion protein

M K S Y N V L P L A Y I S L F L M L F Y
Q V W A Q F P R E C A N I E A L R R G V
C C P D L L P S S G P G T D P C G S S S
G R G R C V A V I A D S R P H S R H Y P
H D G K D D R E A W P L R F F N R T C Q
C N D N F S G H N C G T C R P G W R G A
A C N Q K I L T V R R N L L D L S P E E
K S H F V R A L D M A K R T T H P Q F V
I A T R R L E D I L G P D G N T P Q F E
N I S V Y N Y F V W T H Y Y S V K K T F
L G T G Q E S F G D V D F S H E G P A F
L T W H R Y H L L Q L E R D M Q E M L Q
E P S F S L P Y W N F A T G K N V C D V
C T D D L M G S R S N F D S T L I S P N
S V F S Q W R V V C E S L E E Y D T L G
T L C N S T E G G P I R R N P A G N V G
R P A V Q R L P E P Q D V T Q C L E V R
V F D T P P F Y S N S T D S F R N T V E
G Y S A P T G K Y D P A V R S L H N L A
H L F L N G T G G Q T H L S P N D P I F
V L L H T F T D A V F D E W L R R Y N A
D I S T F P L E N A P I G H N R Q Y N M
V P F W P P V T N T E M F V T A P D N L
G Y A Y E V Q W P G Q E F T V S E I I T
I A V V A A L L L V A A I F G V A S C L
I R S R S T K N E A N Q P L L T D H Y Q
R Y A E D Y E E L P N P N H S M V G G G
G S G G G G S G G G G S M G L V G W G L
L L G C L G C G I L L R A R A Q F P R V
C M T L D G V L N K E C C P P L G P E A
T N I C G F L E G R G Q C A E V Q T D T
R P W S G P Y I L R N Q D D R E Q W P R
K F F N R T C K C T G N F A G Y N C G G
C K F G W T G P D C N R K K P A I L R R
N I H S L T A Q E R E Q F L G A L D L A
K K S I H P D Y V I T T Q H W L G L L G
P N G T Q P Q I A N C S V Y D F F V W L
H Y Y S V R D T L L G P G R P Y K A I D
F S H Q G P A F V T W H R Y H L L W L E
R E L Q R L T G N E S F A L P Y W N F A
T G K N E C D V C T D D W L G A A R Q D
D P T L I S R N S R F S T W E I V C D S
L D D Y N R R V T L C N G T Y E G L L R
R N K V G R N N E K L P T L K N V Q D C
L S L Q K F D S P P F F Q N S T F S F R

N	A	L	E	G	F	D	K	A	D	G	T	L	D	S	Q	V	M	N	L
H	N	L	A	H	S	F	L	N	G	T	N	A	L	P	H	S	A	A	N
D	P	V	F	V	V	L	H	S	F	T	D	A	I	F	D	E	W	L	K
R	N	N	P	S	T	D	A	W	P	Q	E	L	A	P	I	G	H	N	R
M	Y	N	M	V	P	F	F	P	P	V	T	N	E	E	L	F	L	T	A
E	Q	L	G	Y	N	Y	A	V	D	L	S	E	E	E	A	P	V	W	S
T	T	L	S	V	V	I	G	I	L	G	A	F	V	L	L	L	G	L	L
A	F	L	Q	Y	R	R	L	R	K	G	Y	A	P	L	M	E	T	G	L
S	S	K	R	Y	T	E	E	A	K	A	V	Y	N	F	A	T	C	G	I
H	*H*	*H*	*H*	*H*	*H*														

INTERNATIONAL SEARCH REPORT

application No
'GB2005/004754

A. CLASSIFICATION OF SUBJECT MATTER
C12N15/869 A61K39/245

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, MEDLINE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WANG X ET AL: "Cellular immune responses to helper-free HSV-1 amplicon particles encoding HIV-1 gp120 are enhanced by DNA priming" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 21, no. 19-20, 2 June 2003 (2003-06-02), pages 2288-2297, XP004424136 ISSN: 0264-410X Point 1 and 3.3figures 3,4 -/--	1-24

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 February 2006	10/03/2006

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Griesinger, I

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

application No
'GB2005/004754

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HOCKNELL PETER K ET AL: "Expression of human immunodeficiency virus type 1 gp120 from herpes simplex virus type 1-derived amplicons results in potent, specific, and durable cellular and humoral immune responses" JOURNAL OF VIROLOGY, vol. 76, no. 11, June 2002 (2002-06), pages 5565-5580, XP002368987 ISSN: 0022-538X page 5565, right-hand column, paragraph 2	1-24
A	BONNET M C ET AL: "Recombinant viruses as a tool for therapeutic vaccination against human cancers" IMMUNOLOGY LETTERS, vol. 74, no. 1, 15 September 2000 (2000-09-15), pages 11-25, XP002368905 ISSN: 0165-2478 Point 2.1.6 and 6.3tables 4,5	1-24
A	WO 94/21807 A (CANTAB PHARMACEUTICALS RESEARCH LIMITED; INGLIS, STEPHEN, CHARLES; BOU) 29 September 1994 (1994-09-29) abstract	7-9,12, 13
A	WO 98/56919 A (ISIS INNOVATION LIMITED; MCMICHAEL, ANDREW, JAMES; HILL, ADRIAN, VIVIA) 17 December 1998 (1998-12-17) abstract	1-24

INTERNATIONAL SEARCH REPORT

application No.
PCT/GB2005/004754

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-17, 23 and 24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.